Supplemental Document

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High-resolution multi-z confocal microscopy with a diffractive optical element: supplement

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1. FIGURES

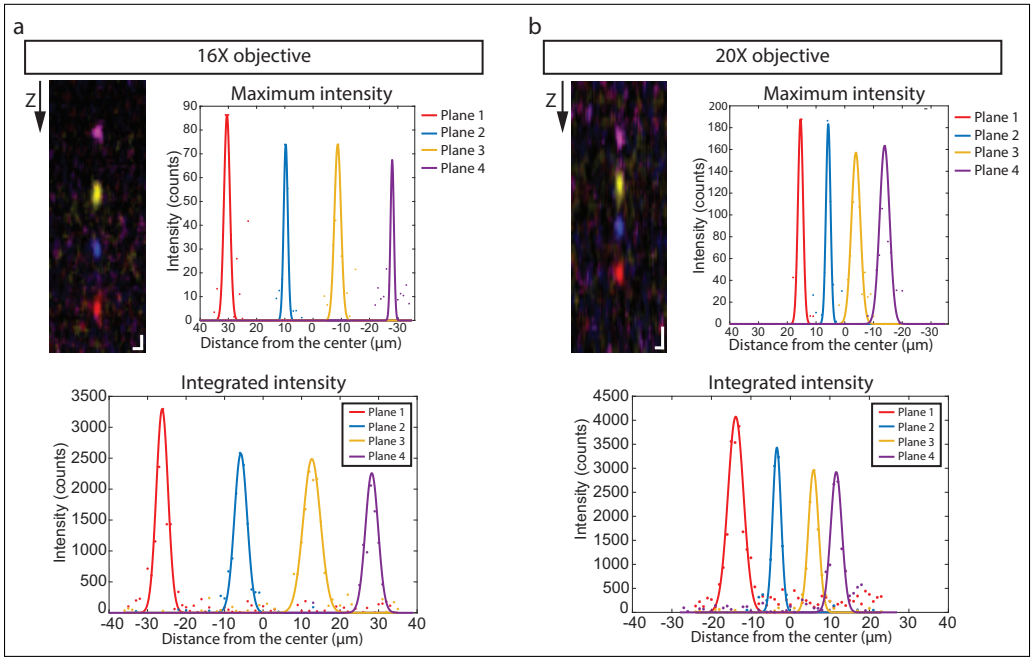

Fig. S1. Raw (un-normalized) intensity produced by a 0.2 μm fluorescent bead when axially scanned through the imaging planes with (a) a 16$\times$ objective (f_{obj} = 12.5mm), and (b) a 20$\times$ objective (f_{obj} = 9mm). Maximum intensity: maximum intensity produced by a single fluorescent bead when axially scanned through the four imaging planes. Integrated intensity: intensity integrated over the x,y plane produced by a single fluorescent bead when axially scanned through the four imaging planes. Average post-objective laser power: 1 mW (for both objectives). Horizontal scale bar: 1 μm. Vertical scale bar: 5 μm. Planes 1-4: deepest to shallowest.

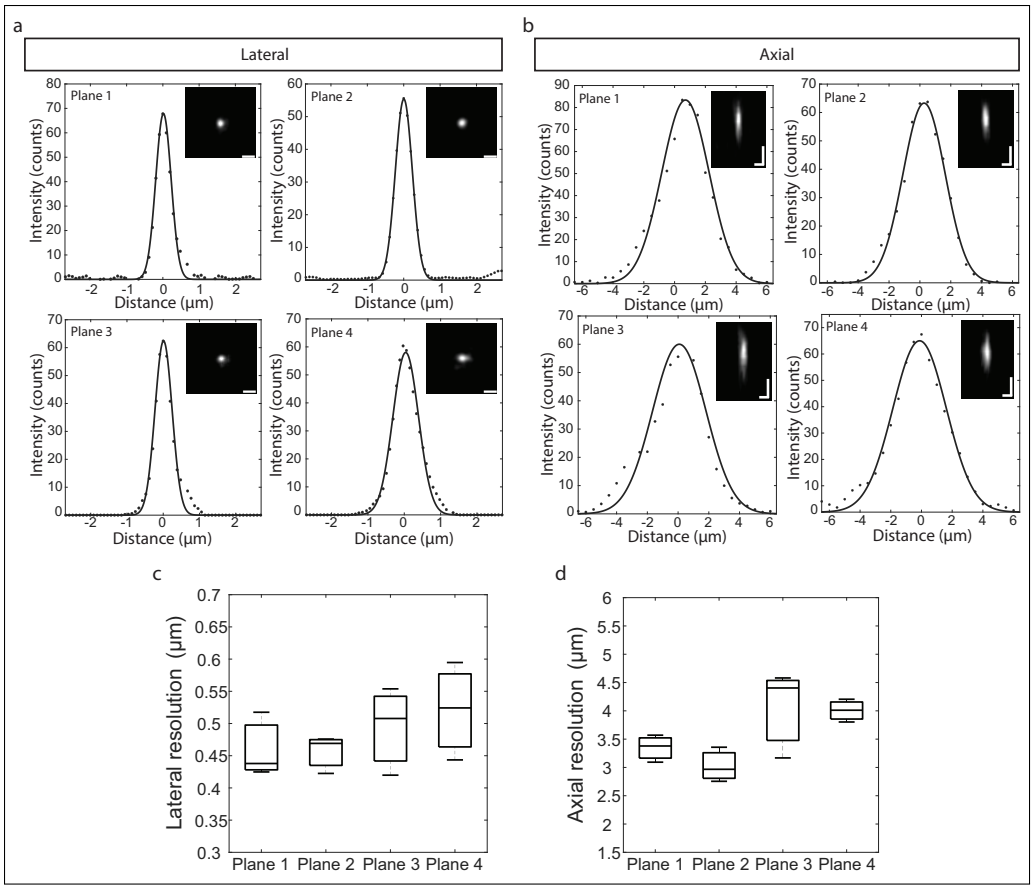

Fig. S2. Lateral (a) and axial (b) intensity produced by a 0.2 μm fluorescent bead through the four planes. Planes 1-4: deepest to shallowest. Horizontal scale bar: 1 μm. Vertical scale bar: 5 μm. Lateral (c) and axial (d) resolution as measured with 0.2 μm fluorescent bead (n = 12). Centerline, medians; limits, 75% and 25%; whiskers, maximum and minimum. Planes 1-4: deepest to shallowest.

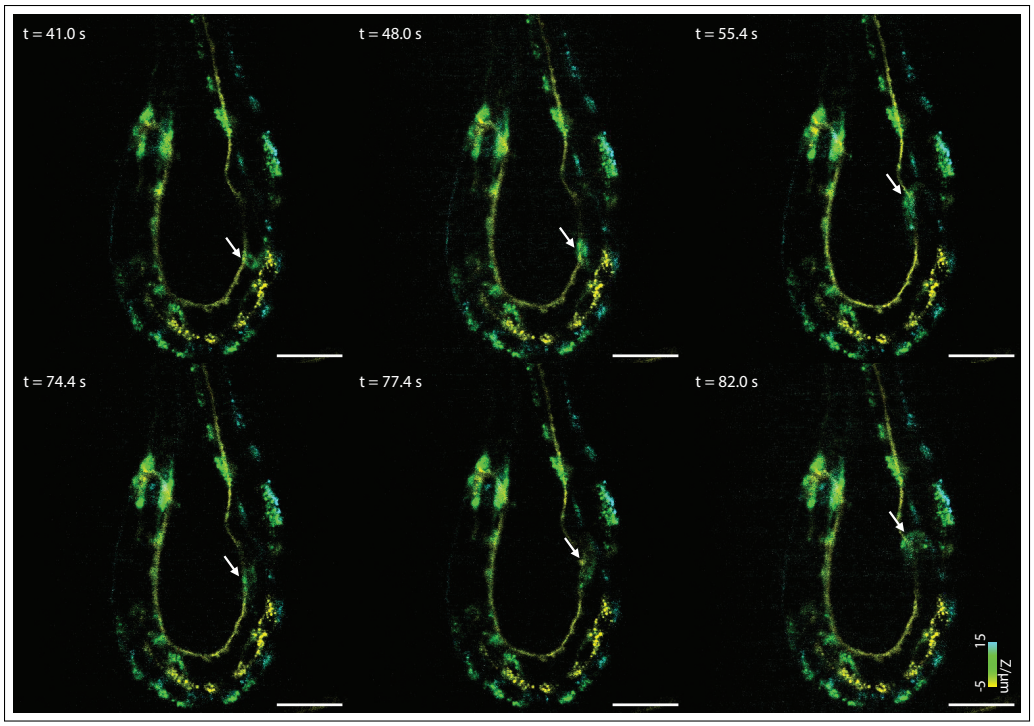

Fig. S3. *In vivo* calcium imaging of *C. elegans* at different times with color corresponding to depth. Scale bar: 50 μm

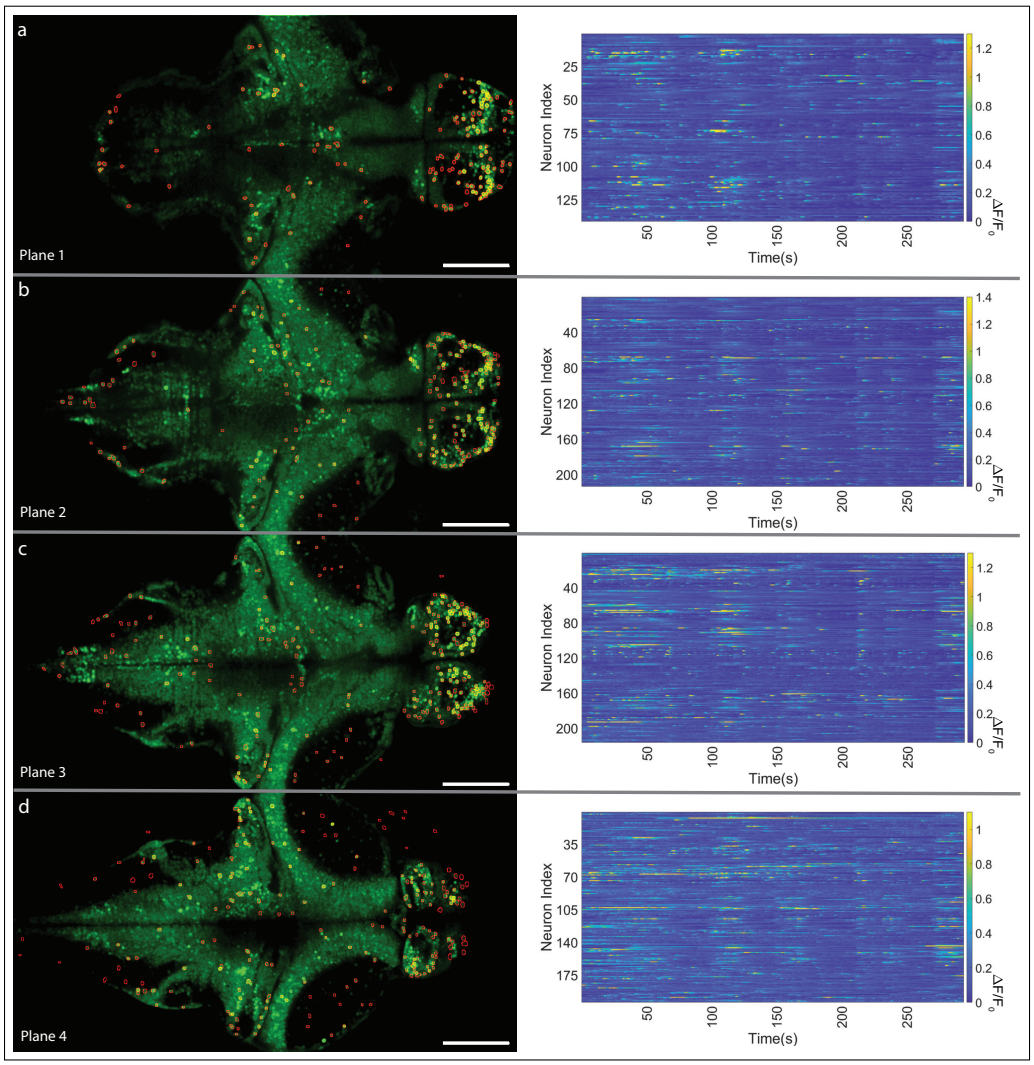

Fig. S4. *In vivo* calcium imaging of zebrafish brain. Planes 1-4: deepest to shallowest. Left: max-min projection from separate planes. Right: activity of identified neurons from separate planes. Scale bar: 100 μm

2. TABLE

Table S1. Description of optical components used

Component	Description	Part number, Vendor
L1	Plano-Convex spherical lens, $f = 25\,mm$, $\phi = 1\,in$	LA1951-A-ML, Thorlabs
L2	Plano-Convex spherical lens, $f = 250\,mm$, $\phi = 1\,in$	LA1461-A-ML, Thorlabs
L3	Plano-Convex spherical lens, $f = 40\,mm$, $\phi = 1\,in$	LA1422-A-ML, Thorlabs
L4	Plano-Convex spherical lens, $f = 30\,mm$, $\phi = 1\,in$	LA1805-A-ML, Thorlabs
L5	Plano-Convex spherical lens, $f = 125\,mm$, $\phi = 1\,in$	LA1986-A-ML, Thorlabs
L6, L8	Achromatic doublets, $f = 100\,mm$, $\phi = 1\,in$	AC254-100-A-ML, Thorlabs
L7	Achromatic doublets, $f = 300\,mm$, $\phi = 2\,in$	ACT508-300-A-ML, Thorlabs
L9	Achromatic doublets, $f = 250\,mm$, $\phi = 1\,in$	AC254-250-A-ML, Thorlabs
SL	Scan lens, $EFL = 70\,mm$	CLS-SL, Thorlabs
DM	Dual-edge laser dichroic beamsplitter, $25.2\,mm \times 35.6\,mm$	Di01-R488/561-25x36, Semrock
GR	Galvo-resonant scanners, $8\,kHz$	LSK-GR08, Thorlabs
DOE	Multifocal diffractive optics element, $\phi = 9.2\,mm$	MF-005-R-Y-A, Holo/or
RP1 - 4	$150\,\mu m$ rounded aperture, $\phi = 1\,in$	214-0688, National Aperture
D1 - 4	Silicon photomultipliers, pixel pitch=$50\,\mu m$, $\phi = 1.5\,mm$	S14420-1550MG, Hamamatsu